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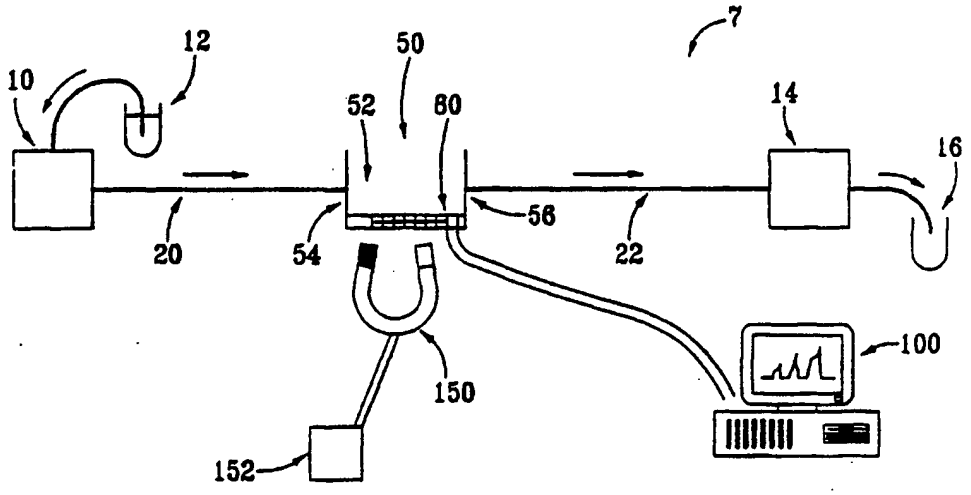
(54) Title: ENZYME-LINKED IMMUNO-MAGNETIC ELECTROCHEMICAL BIOSENSOR



(57) Abstract: A electrochemical biosensor system based on enzyme-linked immuno-magnetic sandwich assay wherein an interdigitated array of electrodes is equipped with a magnet to attract magnetic beads. Magnetic particles bear a first recognition molecule capable of binding to the analyte. Enzymes are chemically modified to complex with the analyte. When the sandwich assay is performed, a substrate is added. The substrate is chosen such that it is cleavable by the enzyme in a reporting molecule capable of redox recycling. A substrate when cleaved preferably leads to a p-aminophenol.

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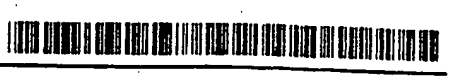

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DESCRIPTION

ENZYME-LINKED IMMUNO-MAGNETIC ELECTROCHEMICAL BIOSENSOR

FIELD OF THE INVENTION

The present invention relates to devices and methods for detecting and quantitating specific analytes in a sample.

BACKGROUND OF THE INVENTION

The detection and quantitation of specific analytes in a sample is in important activity in environmental, health, biotechnology, industrial chemistry and other fields. The assays have also found use in high throughput screening, screening of oligo libraries in the field of functional genomics analysis, combinatorial chemistry screening, and other such fields. The analytes detected or quantitated may be any compound of interest for which there is a specific recognition molecule. Well known recognition molecules include proteins, such as receptors, immuno-globulins, and the like, nucleic acids, their analogs, and the like, haptens, hormones, polypeptides, certain drugs and other such molecules.

Devices and techniques for detecting analytes are well known in the art. These including ELISAs, RIAs, PCR, and the like. Although these techniques have proven very powerful, effective and valuable, they suffer from drawbacks.

Most devices and techniques presently used for the detection of analytes require relatively long reaction

times, complex processes and laboratory conditions. For example, temperatures above room temperature, reaction times in excess of 30 minutes, and strict time limitations. Other drawbacks have included auto-fluorescence of reagents or analytes, in particular in the field of combinatorial chemistry, and when screening small peptide libraries using optical methods.

Decreasing the time necessary to perform an assay while maintaining the precision, sensitivity, reliability and dose-dependent results that can obtained using conventional methods presents great economic advantages, and the patient's well-being in the case of laboratory medicine. The use of an electrochemical sensor rather than an optical sensor further presents other advantages, including avoiding auto-fluorescence and turbidity problems.

SUMMARY OF THE INVENTION

In a first, independent aspect of the present invention, an electrochemical sensor includes an interdigitated array of electrodes on a substantially dielectric substrate and a means for concentrating reagents on the surface of the interdigitated array of electrodes.

In a second, independent aspect of the present invention, an electrochemical reporter system includes a first recognition molecule linked to a magnetic bead, wherein the first recognition molecule can specifically bind an analyte; a second recognition molecule linked to an enzyme, for coupling with specificity the enzyme to the analyte or the first recognition molecule/analyte complex; a substrate, which in the presence of the enzyme is processed into an electrochemical reporter molecule capable of redox recycling; a sensor for detecting the electrochemical

reporter molecule, wherein the sensor has a configuration such that electrochemical reporter molecules, if present, exhibit redox recycling; and a magnetic field generating device positioned such that the magnetic field it generates can attract to the surface of the sensor magnetic beads in solution over the sensor.

In a third, independent aspect of the present invention, an electrochemical reporter device includes a chamber for receiving an analytical reaction having magnetic beads; a sensor on a surface of the chamber, the sensor for detecting electrochemical reporter molecules within the chamber, the sensor having a configuration such that it causes redox recycling of reporter molecules capable of exhibit redox recycling; and a magnetic field generating device capable of generating a magnetic field that attracts magnetic beads present within the chamber onto the sensor.

In a fourth, independent aspect of the present invention, an electrochemical reporter system includes a magnetic bead; a first recognition molecule capable of specifically binding an analyte, the first recognition molecule being linked to the magnetic bead; an enzyme; a coupling element, or second recognition molecule, for coupling with specificity the enzyme to the analyte or the first recognition molecule/analyte complex; a substrate, which in the presence of the enzyme is cleavable into an electrochemical reporter molecule capable of exhibiting redox recycling; a sensor for detecting the electrochemical reporter molecule and having a configuration such that the reporter molecule will exhibit redox recycling; and a magnetic field generating device positioned such that the magnetic beads may be attracted to the vicinity of the sensor.

In a fifth, independent aspect of the present invention, an assay for detecting or quantitating a specific analyte in a sample comprises the following steps: a primary incubation, wherein magnetic beads coated with a recognition molecule that specifically binds an analyte are incubated with a sample; a secondary incubation, wherein the magnetic beads are then incubated with a conjugate comprising an enzyme and a molecule that specifically binds the analyte, or the analyte/recognition molecule complex; capturing the magnetic beads with a magnetic field generating device over a sensor capable of producing redox recycling of an electrochemical capable of undergoing redox recycling; adding a substrate, wherein the substrate in the presence of the enzyme is cleaved into an electrochemical reporter molecule capable of undergoing redox recycling; detecting the presence or measuring the amount of electrochemical present in the solution with the sensor.

In a sixth, independent aspect of the present invention, an electrochemical immunoassay for detecting an analyte in a sample includes the steps of providing an antigen linked to a magnetic bead and an antibody specific for an analyte bound to the antigen, wherein the antibody is coupled to an enzyme or has a coupling element such that it can be specifically coupled to an enzyme; contacting the magnetic bead/antigen/antibody/enzyme complex with a sample to be analyzed; attracting the magnetic bead/antigen/antibody/enzyme complex to the vicinity of a sensor; adding a substrate to the collected magnetic bead/antigen/antibody/enzyme complex, wherein the substrate in the presence of the enzyme is cleaved into an electrochemical reporter molecule capable of exhibiting redox recycling; detecting the presence or measuring the

amount of reporter molecule with the sensor, wherein the sensor is an interdigitated array of electrodes capable of producing redox recycling of the reporter molecule.

In a seventh, independent aspect of the present invention, an electrochemical immunoassay for detecting a specific analyte in a sample includes the steps of providing a recognition molecule linked to a magnetic bead, wherein the recognition molecule is capable of specifically binding the analyte; contacting the magnetic bead with a sample to be analyzed; coupling with specificity an enzyme to the analyte or the recognition molecule/analyte complex; attracting the magnetic bead/recognition molecule/analyte/coupling element-enzyme complex to the vicinity of a sensor with a device capable of generating a magnetic field; adding a substrate, which in the presence of the enzyme is cleaved into an electrochemical reporter molecule capable of exhibiting redox recycling; detecting the presence or measuring the amount of electrochemical with the sensor, wherein the sensor is an interdigitated array of electrodes capable of producing redox recycling of the electrochemical reporter molecule.

In an eighth, independent aspect of the present invention, an electrochemical reporter system includes a magnetic bead; a recognition molecule capable of specifically binding an analyte, the recognition molecule being linked to the magnetic bead; an enzyme; a coupling element, for coupling with specificity the enzyme to the analyte or recognition molecule/analyte complex; a substrate, which in the presence of the enzyme is cleavable into a reporter molecule capable of exhibiting redox recycling; a sensor, for detecting the electrochemical reporter molecule and having a configuration such that the

reporter molecule will exhibit redox recycling; a magnetic field generating device positioned such that the magnetic beads will be attracted to the vicinity of the sensor.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic representation of a device in accordance with the present invention for a detecting and/or quantitating a specific analyte in a sample.

FIG. 2 is a graphic representation of the change in current measured when assaying in accordance with the present invention serum samples having low, medium and high anti-p24 levels.

FIG. 3 is a graph plotting the slope of the kinetic measurement (nA/s) against the original concentration (mIU/ml) of HBsAg in the sample.

FIG. 4 is a dose response curve using electrochemical measurement according to the present invention.

FIG. 5 are measurements of different concentrations using electrochemical measurement with a device without the magnetic beads and magnet of the present invention.

FIG. 6 is a comparison of assays using different number of magnetic beads per sample.

FIG. 7 is a flow chart of methods and devices in accordance with the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Turning in detail to the drawings, FIG. 1 is a schematic representation of a device 7 in accordance with the present invention for detecting and/or quantitating a specific analyte in a sample.

A first pump 10 pumps a processed sample 12 through an inflow tubing, or first tubing segment 20, into the

electrochemical sensing module 50. The first pumping device 10 may be any device that can move fluids containing small particles, or a slurry, through a tubing segment. Preferably, the first pumping device 10 is an adjustable speed peristaltic pump.

The first tubing segment 20 may be any tubing that can carry a fluid having small particles without clogging, or transport a fluid/particulate slurry. It is preferably made from an inert material, *i.e.*, a material that will not interact detrimentally with the fluids and reagents flowing within it. Most preferably, the first tubing segment 20 is TYGON tubing.

The diameter of the first tubing segment 20 will depend on the rate of flow desired. When the first pumping device 10 is a peristaltic pump, the rate of flow of fluids through the tube 20 can be increased by using tubing having a wider inner diameter, or by increasing the speed of the peristaltic pump. Conversely, a slower rate of flow can be achieved by using tubing having a smaller diameter or by decreasing the speed of the peristaltic pump. Preferably the first tubing segment has an inner diameter such that an appropriate rate of flow may be achieved for the specific pump being used. The diameter of the tubing used will also be a function of the size of the beads and the fluid being delivered.

The electrochemical sensing module 50, includes an inflow orifice 54, a chamber 52 for holding the sample, an outflow orifice 56 and a sensor 80. The outflow orifice 56 is connected to the outflow tubing, or second tubing segment 22. The second tubing segment 22 may be connected at its other end to a second pumping (drawing) device 14 (shown in the figure), or alternatively the first pumping device 10

may also be used for this purpose, in which case the system preferably is hermetically sealed.

The second tubing segment 22 advantageously terminates at a waste receptacle 16, collecting chamber, or the like. The properties of the outflow tubing 22 are preferably the same as the inflow tubing 20, described *supra*. If two pumps are used, less flexible, more inert material can be used for the tubing, including TEFLON, or the like.

The electrochemical sensing module 50 may be a disposable, single use unit, in which case the module 50 preferably is adapted to slide and/or snap into and out of the device 7 for easy replacement. Upon sliding and/or snapping into place, the electrochemical sensing module 50 is adapted such that a tight seal is formed between the inflow tubing 20 and the inflow orifice 54, and between the outflow orifice 56 and the outflow tubing 22. Alternatively, the inflow and/or outflow tubing 20, 22 are part of the sensing module 50, and are also discarded and replaced after each use. The waste receptacle 16, collecting chamber, or the like, may also be part of the disposable sensing module 50. The electrical contacts for the IDA are also preferably adapted to plug-in to the controller 100 and/or a power supply once the module slides or snaps into place.

The sensor 80 may be any device that can detect and/or measure an electrochemical reporter that can undergo redox recycling, while providing for redox recycling of the electrochemical reporter. See, for example, WO 99/07879 and United States Patent No. 5,670,031. Preferred is an interdigitated array of electrodes (IDA) with a spacing between the electrodes of about 800μm, or smaller. Most preferred is an array of electrodes with a spacing between

the electrodes between about 200μm and about 400μm, for example, an array of electrodes with a spacing between the electrodes of about 300μm.

The sensor 80 may have one or more IDAs. More IDAs provide for greater sensitivity, but are not necessarily indispensable, depending on the assay. When more than one array is present, (i.e., a "ganged" IDA sensor) the arrays may be linked in series or in parallel. Independent combinations thereof may also be used.

The sensor 80 is linked to a system controller 100 including a multipotentiostat that provides a specified potential across the IDA or IDAs and measures the dose dependent current resulting from redox recycling of electrochemical reporter molecules proximal to the IDA. Alternatively, the information may be derived by scanning voltammetry, or the like. The system controller is thus capable of measuring and preferably also recording the change in voltage, and/or current, and the like, in the IDA. If more than one IDA is present in series, the system controller 100 preferably can measure and also record the change occurring in each independent IDA or the sum of such changes. The system controller may advantageously be part of a computer network, such that processes and results can be order, monitored, controlled, retrieved and/or analyzed remotely.

A magnetic field generating device 150, or the like, is positioned relative to the electrochemical sensing module 50 and is capable of generating a magnetic field of such strength that when a fluid having magnetic beads is circulated within the chamber 52, a quantity of magnetic beads adequate for the detection or quantitation of the

analyte of interest will be attracted onto the sensor's 80 surface.

The magnetic field generating device 150 may be activated/deactivated by an on/off switch 152, or the like. The switch may be under the control of a system controller 100, or the like.

Alternatively, the magnetic field generating device 150 may be a permanent magnet. In that case it is preferable that the magnet be moveable such that in at least a first position the magnetic field it generates affects magnetic particles within chamber 52, such that it may cause magnetic beads to be attracted onto the sensor surface 80. When the magnet is moved into a second position the magnetic field does not significantly affect magnetic particles within the chamber 52, such that the magnetic beads are no longer attracted onto the sensor surface 80, to facilitate clearing the magnetic beads from the sensor after the detection and/or quantitation of analyte has been achieved. An activatable/deactivatable magnetic field generating device may be used, but is not necessarily required, when a single use/disposable electrochemical sensing module 50 is used.

In use, a buffer is pumped through the system and over the sensor 80 to establish a baseline. The buffer is flowed over the sensor 80 at any effective rate, however, a slow rate (about 0.2mL/min) is preferred. Any effective buffer may be used, but enzyme substrate buffer (ESB), described below, is preferred.

The magnet 150 under the sensor 80 may be activated 152, or, alternatively, a magnet may be placed under the sensor 80, at any time prior to the flow of the sample including the magnetic beads over the sensor 80. The magnet 150 should be able to generate an applied field such that an

adequate amount of magnetic beads may be drawn and captured on the surface of the sensor 80.

Next, the processed sample to be tested 12 is circulated over the sensor 80. The processed sample may be prepared by any effective method. One method for preparing a processed sample is described in more detail *infra*. In general, the processed sample includes magnetic beads, or the like, and an enzyme indirectly linked to the magnetic beads by the analyte, the recognition molecule or the recognition molecule/analyte complex.

The processed sample may be circulated over the sensor 80 surface for any effective amount of time, preferably until an adequate quantity of beads is captured by the magnet 150 over the sensor 80 surface. Preferably, the bead solution is circulated for approximately 2 minutes at medium to fast flow rates (approximately 0.38mL/min). The effect of the magnet 150 and the flow rate should be such that an adequate concentration of beads is captured over the sensor surface 80.

A substrate is then circulated over the sensor 80. The substrate may be circulated at any effective rate, however, a slow flow rate (approximately 0.2mL/min) is preferred. The flow is then preferably stopped while substrate solution is over the sensor 80 and the signal is measured and/or recorded by the system controller 100, with no flow, for the desired period of time.

The signal may be measured for any adequate amount of time. In general, however, the signal may be measured for about 90 to about 100 seconds or about 60 seconds of useable data. Longer or shorter measurements may be used if necessary. It is within the skill in the art to determine

the optimal length of time the measurement should take place for a given set of conditions and samples.

The substrate will depend on the enzyme and the conditions used. Any effective substrate may be used. A non-exclusive list of enzyme/substrate pairs that may be used in accordance with the present invention is disclosed in WO 99/07879. Any effective concentration of substrate may be used. The preparation of the preferred substrate solution is described in greater detail *infra*.

Once the sample has been assayed, the beads may be cleared from the sensor 80 surface by deactivating 152, or removing, the magnet 150 from the proximity of the sensor 80, and circulating fresh buffer at a sufficiently rapid rate of flow over the sensor 80.

Alternatively, if a disposable sensor is being used, once the sample has been assayed the sensor module 50 may be removed and discarded.

In case the magnetic beads are to be cleared from the sensor, any effective buffer may be used, but ESB is preferred. The flow rate is preferably about 0.43mL/min. The addition of bubbles to the buffer flow has been found to assist the clearance of the beads. The washing buffer may be applied for any effective amount of time, however, generally between about 45 and 60 seconds has been found to be sufficient. Once the beads are washed out, fresh buffer may be recycled over the sensor 80 until the baseline equilibrates again. This step generally takes about 30 seconds. The sensor 80 is then ready for a new sample.

Another aspect of the present invention is a fast and reliable assay for measuring and quantitating analytes in a sample. The method is particularly effective when used with the device of the present invention.

Analytes that may be detected or quantitated include any compound of interest for which there is a specific recognition molecule. Well known recognition molecules include proteins, such as receptors, immuno-globulins, and the like; nucleic acids, their analogs, and the like; haptens; hormones; polypeptides; certain drugs; and other such molecules.

In general, the assay uses magnetic beads, or the like, which are commercially available. Any effective magnetic beads may be used, however Tosyl-activated DYNABEADSM-450 (DYNAL Inc, 5 Delaware Drive, Lake Success, NY 11042 Prod No. 140.03, 140.04,) or the like, are preferred. The magnetic beads may be of any size that can be held to the chip surface with a magnetic field.

The magnetic beads are generally coated with a recognition molecule that binds with specificity and high affinity to the analyte to be detected or quantitated. Methods for coating magnetic beads with specific recognition molecules are well known in the art. The magnetic beads are generally coated by dissolving the coating material in carbonate buffer (pH 9.6, 0.2 M) or the like, or any other well known in the art method.

For the DYNABEADS, the instructions provided by the manufacturer may be used. Briefly, the magnetic beads are first resuspended and homogenized by vortexing, or the like, and a volume corresponding to the number of beads desired is pipetted into a test tube. The magnetic beads are concentrated using a magnet, and the supernatant is pipetted off, leaving the magnetic beads undisturbed.

The beads are then resuspended in an ample volume (preferably greater than original volume) of any effective buffer. It is within the skill in the art to determine the

most effective buffer for the recognition molecule to be used. Buffers that may be used include, for example, phosphate buffer pH 7.4, borate buffer pH 9.5 or acetate buffer pH 4.0 with molarities between 0.1M and 0.5M.

The beads are mixed gently with the final coating solution for any effective period of time. Generally, the beads are mixed with the final coating solution for about 2 minutes.

The magnetic beads are once more concentrated with a magnet, and the supernatant pipetted off leaving the beads undisturbed. The beads are then resuspended in an appropriate volume of any effective buffer. Effective buffers include, among other buffers, phosphate buffer pH 7.4, borate buffer pH 9.5 or acetate buffer pH 4.0. The beads are now ready for coating.

For coating, the magnetic beads are thoroughly resuspended in any effective buffer. Effective buffers include, among other buffers, phosphate buffer pH 7.4, borate buffer pH 9.5 or acetate buffer pH 4.0. From between about 1µg to about 10µg of the pure recognition molecule, if it is a protein, polypeptide or the like, per 10^7 magnetic beads may be added to the magnetic bead/buffer solution. Preferably, about 5µg of the pure recognition molecule, if it is a protein, polypeptide or the like, per 10^7 magnetic beads is added to the magnetic bead/buffer solution. The solution is then vortexed for 1-2 minutes. The manufacturer of DYNABEADS recommends a concentration of 4-10 x 10^8 DYNABEADS per ml final coating solution (including the antibody or other recognition molecule).

Preferably the salt concentration in the final coating solution is greater than about 0.05M. Higher pH and/or higher temperature will give a quicker formation of chemical

bonds. The upper pH and temperature limit is determined based on the recognition molecule used to coat the magnetic beads.

The magnetic beads/recognition molecule solution may then be incubated for 16-24 hours at 37°C with slow tilt rotation, or the like. Lower temperatures may be used for temperature sensitive recognition molecules. Higher temperatures and shorter incubation times may be used for stable recognition molecules. Preferably the magnetic beads are not permitted to settle during the incubation period.

Phosphate buffer pH 7.4 (0.1M) may be prepared by dissolving 2.62 g $NaH_2PO_4xH_2O$ (MW 137.99) and 14.42 g $Na_2HPO_4x2H_2O$ (MW 177.99) in distilled water and adjusting the volume to 1000 ml.

Borate buffer pH 9.5 (0.1M) may be prepared by dissolving 6.183 g H_3BO_3 (MW 61.83) in 800 ml distilled water, adjusting the pH to 9.5 using 5M NaOH and then adjusting the volume to 1000 ml with distilled water.

Acetate buffer pH 4.0 (0.1M) may be prepared by dissolving 2.86 ml acetic acid (CH_3COOH), in 900 ml distilled water, adjusting the pH to 4.0 using 5M NaOH and adjusting the volume to 1000 ml with distilled water.

These buffers may be used for prewashing and coating of DYNABEADSM-450 Tosylactivated. It is preferred that no proteins, sugars, or the like be added to these buffers.

Recognition molecules other than proteins or polypeptides may also be directly or indirectly used to coat the magnetic beads. For example, nucleic acids and their analogs can be attached to the magnetic beads by an avidin biotin link, or the like; by binding the nucleic acid or analog to a protein like albumin or the like, which is then used to coat the magnetic beads; or by other methods well

known in the art. Other recognition molecules, including hormones, haptens, sugars, polypeptides and the like may similarly be bound to the magnetic beads using strategies well known by those of skill in the art.

After the incubation with the coating solution, the magnetic beads are concentrated using a magnet, and the supernatant is pipetted off. The coated beads are then washed, preferably a total of four times. Twice in buffer D for 5 minutes at 4°C, once in buffer E for 24 h at 20°C or for 4 hours at 37°C, and once in buffer D for 5 minutes at 4°C. The beads should be coated and ready for use after this procedure. The amount of specific recognition molecules bound to the beads may be established by radioactive labeling, immunofluorescent methods, spectrophotometry, or any other method known in the art.

The beads may be stored in buffer D at 4°C, usually for months, depending on the stability of the immobilized material. If the beads are stored for more than two weeks, it is preferred that they be washed twice in PBS/BSA for five minutes before use.

Buffer D consists generally of PBS pH 7.4 with 0.1% w/v bovine serum albumin (BSA) or human serum albumin (HSA). It may be made by dissolving 0.88g NaCl (MW 58.4) and 0.1% (w/v) BSA or HSA to 80ml 0.01M Na-phosphate pH 7.4 (see above). The solution is then mixed thoroughly and the volume adjusted to 100 ml with 0.01M Na-phosphate pH 7.4.

Buffer D is generally used for washing precoated DYNABEADS. According to the manufacturer, this buffer or any buffer containing protein or amino-groups (glycine, Tris etc.) should preferably not be used for pre-washing or coating of DYNABEADSM-450 Tosylactivated.

If a preservative is needed in the coated product, an effective amount of sodium azide (NaN_3) may be added to buffer D. Preferred is a final concentration of 0.02% (w/v). This preservative is cytotoxic and should be carefully removed before use by washing. Required safety precautions should be followed when handling this material.

Buffer E: 0.2M Tris pH 8.5 with 0.1% (w/v) BSA (HSA), may be made by dissolving 2.42g Tris in 80 ml distilled water, and adjusting the pH to 8.5 using 1 M HCl, then dissolving 0.1% BSA/HSA and adjusting the volume to 100ml.

All reagents should preferably be analytical grade.

To test a sample for the presence or quantity of an analyte, the sample in which the analyte is to be detected or quantitated is combined with the coated beads in a primary incubation. The primary incubation in general consists of adding the sample to be analyzed to the magnetic beads pre-coated with a first recognition molecule. In general, the volume in which the primary incubation is carried will depend on the number of beads to be used and the final volume at which the reaction will take place.

Any effective number of beads per volume may be used in the primary incubation. The desired number of beads coated with the appropriate recognition molecule are pipetted and then washed in modified buffer E (MBE), which consists of 0.2 m Tris buffer, pH 8.5, with 1.0% (w/v) BSA, and are then resuspended in the desired volume of MBE. In general between about $4\text{-}5x10^4$ and about $4\text{-}5x10^{10}$ beads in 20µl may be used for an assay having a final volume of 40µl. Preferably, between about $4\text{-}5x10^5$ and about $4\text{-}5x10^7$ beads in 20µl may be used for an assay having a final volume of 40µl. Most preferred is the use of between about $4\text{-}5x10^6$ and about

$1x10^7$ beads in 20µl for an assay having a final volume of 40µl.

Any sample generally tested using conventional techniques may also be tested using the methods and devices of the present invention. The sample may be diluted in MBE if necessary. In general, for example, it has been found that serum samples may be diluted 1:2 or 1:4, or even greater, if the analyte is present in sufficient concentrations. Diluting the sample has been found to decrease the background.

The sample and the beads are mixed, generally in a 1:1 (v/v) ratio. Preferably, 20µl of beads and 20µl of sample are mixed, for a total reaction volume of 40µl.

Several experiments have been performed in which the primary incubation time period was examined, with time incubation time intervals ranging from about 0.5 of a minute to about 30 minutes. Although longer incubations were found to yield more sensitive results, in general primary incubations of about 10 minutes or less were found to yield highly sensitive results. Primary incubations of about 5 minutes or less were also found to yield highly sensitive results. Most preferred are primary incubation of between about 1 and about 2 minutes.

After the primary incubation the beads are preferably washed twice with MBE (100µl per 40µl in the primary incubation may be used).

The secondary incubation with a conjugate, generally a second recognition molecule that specifically binds the analyte (or the first recognition molecule/analyte complex) and is conjugated or may be conjugated to an enzyme, is then effectuated. Other methods, for example, complementation of polypeptide fragments of *beta*-galactosidase, or the like,

may also be used. Any effective amount and concentration of the conjugate or second recognition molecule may be used. Preferably, however, the secondary incubation takes place in the same volume as the primary incubation. The conjugate may be diluted in MBE, as necessary.

Secondary incubations ranging in time from about 0.5 of a minute to about 30 minutes were tried. Although longer incubations were found to yield more sensitive results, in general secondary incubations of about 10 minutes or less were found to yield highly sensitive results. Secondary incubations of about 5 minutes or less were also found to yield highly sensitive results. Most preferred are secondary incubations of between about 1 and about 2 minutes. The solution is preferably gently rocked during the procedure to ensure mixing of the reaction components.

It was found that when the systems and methods of the present invention are used, the primary and secondary incubations may be performed at room temperature (17°C - 25°C), with excellent results. Higher or lower temperatures may be used if appropriate.

After the secondary incubation the liquid phase may be discarded and the reaction washed. In general, the reaction is washed three times in PBS with 0.05% TWEEN 20. Prior to injection into the device, the reaction is washed with Enzyme substrate buffer (ESB), (0.1 M Phosphate, 0.1 M NaCl, pH 6.8), and the reaction resuspended in ESB. In general, with the device described above, the reaction is resuspended in 200µl.

The substrate to be used will depend on the enzyme in the conjugate. In general, if the enzyme is beta-galactosidase, an effective substrate is P-aminophenyl-beta-D-galactopyranoside (PAPG). A concentration of 2mM is

preferred. A non-exclusive list of enzymes and substrates is disclosed in WO 99/07879.

Other assay formats known in the art may also be adapted for use in accordance with the present invention. See, e.g., WO 99/07879.

Yet another embodiment of the present invention is a kit including reagents to perform the assays of the present invention. The kit may include any combination of reagents used in performing the assays. It may include, for example, a first vial or the like having magnetic beads pre-coated with a recognition molecule for the analyte of interest; a second vial or the like having a second recognition molecule, specific for the analyte or the first recognition molecule/analyte complex, the second recognition molecule being conjugated or conjugatable to an enzyme; a substrate, which in the presence of the enzyme generates an electrochemical capable of redox recycling. The kit may also contain a single use electrochemical sensor module. Preferably the kit also includes buffers, positive controls, negative controls, and other reagents for use in the assay.

EXAMPLES

Experiments were conducted to evaluate faster, more sensitive devices and methods for detecting and quantitating analytes based on the proportional production of an electrochemical capable of undergoing redox recycling and the measurement of the electrochemical with an IDA having a conformation such that the electrochemical will undergo redox recycling.

Unless otherwise specified, the following materials were used. M450 Tosyl-activated magnetic beads (Dynal, Product No. 140.04). Coating buffer 0.1 M Phosphate Buffer Saline (PBS), pH 7.4. Post-coating washing buffer PBS, pH

7.4 with 0.1% (w/v) bovine serum albumin (BSA) (1x crystallized, Sigma, cat# A-4378). Storage buffer, PBS, pH 7.4 with 0.1% (w/v) BSA and 0.02% (w/v) sodium azide. Tosyl blocking buffer, 0.2 M Tris Buffer, pH 8.5, with 0.1% (w/v) BSA. Recombinant HIV-1 p24 antigen (Devaron, Inc., cat# 301-8-2, clone # AR-DEV). Human serum derived hepatitis B surface antigen AD subtype (adHBsAg) (Genzyme Diagnostics, Cat# ABH0707, Lot#M-22975). Recombinant HBsAg (ayw subtype) (Genzyme Diagnostics, Cat#ABH0705, Lot# M-22756). Goat anti-human (IgG H+L-specific) conjugated to beta-galactosidase (American Qualex, cat# A110GN, lot# GG017). P-aminophenyl-beta-D-galactopyranoside (Sigma, cat# A-9545) at 2mM, in enzyme substrate buffer (0.1 M Phosphate, 0.1 M NaCl, pH 6.8).

Modified buffer E (MBE), 0.2 m Tris buffer, pH 8.5, with 1.0% (w/v) BSA, is used in the coating to block the unbound, active tosyl groups. It has been found that by using Tris buffer with BSA, the assay is less likely to produce non-specific binding.

The experiments were performed with the following positive and negative controls. Human serum with antibody to p24: negative α-p24 (98-058-08445), approximate titer of 0; low + α-p24 (98-053-01456), approximate titer of 261; medium + α-p24 (98-062-07940), approximate titer of 1,515; and high + α-p24 (98-058-07537), approximate titer of 104,186. Human serum with antibody to HBsAg: high + α-HBsAg (98-306-04981), approximate concentration of 4742 mIU/ml; negative α-HBsAg (98-306-05415). Dilutions of high + α-HBsAg with the negative serum were used to produce samples with lower α-HBsAg titers.

A colorimetric assay was performed on the samples for comparative purposes. This colorimetric assay is a widely utilized non-electrochemical detection technique. For the colorimetric bead optical endpoint assays, peroxidase-Affinipure F(ab) fragment mouse anti-human IgG Fc (gamma) fragment specific (Jackson Immunoresearch, code 209-036-098, lot 25206) was used and OPD was obtained from Abbott kit products (OPD tablets no. 7181E, OPD diluent no. 5695).

Example 1

The desired number of beads were washed in MBE and resuspended in the desired volume of MBE. In particular, 4-5x10^6 magnetic beads were used for an electrochemical reaction, while 1x10^6 beads were used in the optical reaction.

The primary incubation in general consists of adding the sample to be analyzed to beads (20µl) pre-coated with the recognition molecule. For the test indicated below, the serum sample was diluted 1:4 in MBE (20µl per well) for a total reaction volume of 40µl.

Several experiments have been performed in which the primary incubation time period was examined, with time incubation time intervals ranging from about 0.5 of a minute to about 30 minutes. Although longer incubations were found to yield more sensitive results, in general primary incubations of 1-2 minutes were found to yield a high sensitivity.

The reaction was then washed twice with MBE (100µl) and the beads incubated (secondary incubation) in goat-anti-human beta-galactosidase conjugate (40µl per well, 1:1000 dilution in MBE). Incubations ranging in time from about 0.5 of a minute to about 30 minutes were tried. Although longer secondary incubations were found to yield more

sensitive results. In general, however, incubations of 1-2 minutes were found to yield a high sensitivity. The solution is preferably shaken during the procedure to ensure mixing of the reaction components.

It was found that when the systems and methods of the present invention are used, the primary and secondary incubations may be performed at room temperature (17°C - 25°C), with excellent results.

After the secondary incubation, the liquid phase was discarded and the reaction washed three times (100µl) in PBS with 0.05% TWEEN 20 (PBST). The reaction was then washed once (100µl) with ESB, and the reaction resuspended in ESB (200µl).

The sensor (a single array of an interdigitated array of electrodes, as generally described in United States Patent No. 5,670,031) was activated and ESB flowed over the sensor at a slow rate (about 0.2mL/min) until a stable baseline was achieved. The magnet was then placed under the sensor. The magnet was placed such that it generated a field of force sufficient to capture magnetic beads on the surface of the sensor.

The processed sample was then circulated over the sensor. The bead solution was circulated for approximately 2 minutes at medium to fast flow rates (approximately 0.38mL/min). Due to the magnet, a high concentration of beads was captured over the sensor surface.

The substrate (2mM PAPG, 100µl) was then circulated over the sensor at a slow flow rate (approximately 0.2mL/min). The flow was then stopped while substrate solution was over the sensor and the signal was measured with no flow for the desired period of time. The signal was

measured for about 90 to about 100 seconds for 60 seconds of useable data.

The beads were cleared from the sensor by removing the magnet from the proximity of the sensor and circulating fresh ESB at a high flow rate (approximately 0.43mL/min) over the sensor. The addition of bubbles to the ESB flow was found to assist the clearance of the beads. The washing step generally took between about 45 and 60 seconds. Once the beads were washed out, fresh ESB was recycled over the sensor until the baseline equilibrated. This step generally took about 30 seconds. The sensor was then ready for a new sample.

After coating the magnetic beads with p24, the beads were incubated for one minute with the serum to be tested (primary incubation), washed, incubated for one minute with goat anti human beta-galactosidase IgG (secondary incubation) and washed. The procedure described above was then used to concentrate the beads over the sensor and the substrate was added.

Figure 2 is a graphical representation of the measured change in voltage over time. The first peak, starting at about t 11900 and ending at about t 12200 corresponds to the measurement of anti p24 in the low titer serum. The second peak, starting at about t 12500 and ending at about t 12800 corresponds to the measurement of anti p24 in the medium titer serum. The third peak, starting at about t 13200 and ending at about t 13500 corresponds to the measurement of anti p24 in the high titer serum.

The average slope was calculated from the data graph (nAmp = y-axis; time (seconds) = x-axis) for data points acquired from the 20th second through the 100th second of measurement. Data was acquired at the rate of 2

observations per second and recorded as spreadsheet entries by the acquisition program (Origin Software). For the low titer serum the average slope was estimated to be 0.061, the average slope for the medium titer serum was estimated to be 0.112, and the average slope for the high titer serum was estimated to be 0.344. These values can be compared to the optical measurements obtained using a commercially available kit. The optical measurement provided values of 0.147, 0.291 and 0.495 for the low, medium and high titer serums respectively. Advantageously, a tight correlation therefore was observed between the results obtained using the present invention and those obtained using commercially available systems. The results obtained using the present invention, however, required only a fraction of the time required for the commercially available system and method.

Example 2

An experiment was conducted to find out the sensitivity of the systems and methods of the present invention under the conditions described below. Serial dilutions of human serum having concentrations equivalent to 0, 15, 50, 100, 200, 400 and 800 mIU/ml anti-HBsAg were prepared. Magnetic beads (DYNABEADS M450) which had previously been coated with HBsAg were washed and resuspended in MBE.

For the primary incubation, the serum samples were diluted 1:1 with MBE, and 25µl of each diluted sample was dispensed in a microtiter plate well. $5x10^6$ coated beads in 25µl MBE were then added to each well. The samples were incubated for 2 minutes with gentle rocking at room temperature. The samples were then washed twice with MBE.

For the secondary incubation, 50µl of a 1:1000 dilution of goat anti human beta galactosidase conjugate in MBE was added to each well. The samples were incubated for 2

minutes with gentle rocking at room temperature. The samples were then washed twice with MBE, twice with PBST, once with ESB, and then resuspended in 250µl. The samples were then individually loaded onto the chip. PAPG 2mM in ESB was then added to the system and the voltage in the sensor recorded.

Figure 3 is a graph plotting the slope of the kinetic measurment (nA/s) against the original concentration (mIU/ml) of HBsAg in the sample. The results indicate a correlation having an R^2 equal to 0.8626. Qualitative results are obtainable for concentrations at least as low as 15 mIU/ml, with semi-quantitative results obtainable from 50 mIU/ml or greater under these conditions. As shown in Example 4 *infra*, more sensitive results may be obtained by slightly varying the conditions.

Example 3

In this set of experiments, hepatitis B surface antigen (HBsAg) levels in human serum were measured. The measurement of dilutions corresponding to 0, 15, 50, 100, 200, 400 and 800mIU/ml were obtained using side by side matched conditions for all reagents. A direct comparison was made between the sensitivity of the methods and devices in accordance with the present invention and the devices and methods disclosed in WO 99/07879, which are at least as sensitive and reliable as the colorimetric assay commercially available, to obtain a direct comparison between the system and method with and without the novel aspects of the present invention.

Preliminarily, DYNABEADS(M450) ($4x10^8$) were coated with 200µg of HBsAg (100µg of ad subtype obtained from human plasma and 100µg of ayw recombinant HBsAg) in a 850µl reaction volume following the same protocol used for p24 in

Example 1. In parallel, a substantially identical surface area of a microtiter plate was also similarly coated.

Sets of microbeads and microtiter plate wells were then incubated (primary incubation) with the different dilutions of HBsAg serum samples for two minutes at room temperature. After two minutes the samples were removed and the different sets of microbeads and the wells of the microtiter plates were washed. The microbeads and microtiter plate wells were then subjected to a two minutes secondary incubation with goat anti-human β-galactosidase. The conjugate was then removed and excess conjugate was washed off.

The microbead samples corresponding to the different dilutions of the sample were then individually measured by capturing the microbeads over the sensor, adding the substrate solution and measuring the change in voltage over a period of 60 seconds. Figure 4 is graphic representation of the results obtained.

Similarly, the matched pairs measured in the microtiter plates were assessed by stopping the reaction after two minutes, and measuring in a traditional manner the electrochemical generated. Figure 5 is a graphic representation of the results obtained.

As can be seen by comparing figure 4 to figure 5, the method and device of the present invention provides under these conditions a linear dose response that can qualitatively detect as low as 200mIU/ml anti-HBsAg at 2 standard deviations uncertainty, with a linear dose response up to 800 mIU/ml. In contrast, the results obtained using the traditional method showed no statistically significant difference between the samples, i.e., the traditional method under these conditions does not demonstrate a measurable dose dependent increase in electrochemical, and in fact the

traditional method under these conditions cannot qualitatively detect the analyte at a concentration below 300 mIU/ml.

The sensitivity and reliability of the method and device, in particular as demonstrated by the results obtained using the short primary and secondary incubations at room temperature was much greater than expected. These properties of the methods and devices of the present invention are valuable because they permit faster, cheaper, less cumbersome analysis of a sample.

Example 4

Having determined the unexpected and valuable properties of the system and method of the present invention, experiments were performed in order to optimize the procedure. In this experiment, the effect of the concentration of magnetic beads per sample to be analyzed was evaluated.

The experiment was generally set up as in Example 2. Serum samples having three concentrations (100 mIU/ml, 500 mIU/ml, 2000 mIU/ml) of HBsAg were tested using either 800,000 beads per sample, as in Example 2, or 5,000,000 beads per serum sample. The data was derived as in Example 2, and is shown graphically in Figure 6.

The data from the experiment indicates that the sensitivity of the device and method can be further greatly enhanced by increasing the number of magnetic beads per volume of sample. This presents a further advantage over the traditional method since it permits an increase in the surface area over which reactions can take place. As may be seen from Figure 6, a concentration of 100 mIU/ml can easily be detected using the larger amount of beads. Lower concentrations were not tested, but the linearity of the

response indicates that concentrations as low as 15 mIU/ml should be easily obtained with the increased number of beads.

As shown in Fig. 7, another embodiment of the invention comprises forming linearly disposed discrete solution compartments within a conduit. Each solution compartment may be defined by interposing a separator, such as a gas bubble, within a carrier fluid at predetermined points. In this manner, the carrier fluid may be divided up into solution compartments, each of which is formed or sandwiched between two opposing gas bubbles within the conduit. The conduit, such as an inert tube, may be parallel to a ground surface, vertical to a ground surface, or even at an angle thereto. Preferably, the conduit is vertically positioned relative to a ground surface.

Each solution compartment may contain a different composition of materials, such as a sample or a conjugate, to respectively define a sample solution compartment or a conjugate solution compartment. At least one of the solutions compartments contains an attractable bead coated with a recognition molecule to define a coated bead solution compartment.

In operation, each of the solution compartments are transported over time, from left to right as seen in Fig. 7, within the conduit via a peristaltic pump or the like. An attraction device, such as a magnet, electromagnet, or the like, is disposed about the conduit. The attraction device, when actuated, is capable of attracting one or more of the attractable beads for processing/testing. The attraction device preferably contains a sensor, or IDA chip as described in detail *supra*. The sensor is capable of measuring the manipulated and processed beads after they

have been transported through the conduit and/or subjected to the "conveyor belt" of discrete solution compartments.

Advantageously, as each of the solution compartments are transported within the conduit, due to the placement of the attraction device, the attraction device is capable of selectively retaining at least some of the attractable beads within the conduit. In this manner, the attracted attractable beads are effectively separated from the carrier fluid. As the carrier fluid continues to flow through the conduit, the next linearly disposed solution compartment can manipulate the temporarily restrained beads. For example, if the next linear solution compartment comprises a wash solution, the temporarily restrained beads will be washed. Similarly, if the solution compartment preceding the wash solution compartment contains a substrate/carrier fluid, the bathed and temporarily restrained beads can be subjected to the substrate/carrier fluid within the conduit.

As is apparent to one of ordinary skill in the art, such a conduit arrangement allows for the implementation of separate processing steps in an endless sequence that can be manipulated depending on the assay. The preferred linear order of the solution compartments, as illustrated in Fig. 7 from left to right, is as follows: a substrate/carrier fluid compartment, a conjugate/carrier fluid compartment, a sample/carrier fluid compartment, and a bead compartment. Most preferably, a wash solution compartment separates each of the four identified material-containing solution compartments. Multiple attraction devices are also preferably used to facilitate improved processing techniques.

In sum, the preferred operational steps of this embodiment of the invention, as illustrated in Fig. 7, are

as follows: (1) transporting a coated bead solution compartment to a first separation station having an actuatable attraction device; (2) actuating the attraction device to attract some of the coated beads in the coated bead solution compartment such that some of the coated beads are temporarily restrained within the first separation station and separated from the carrier fluid; (3) flowing a wash solution compartment into the first separation station; (4) flowing a sample/carrier fluid solution compartment over the attracted beads; (5) actuating the attraction device to release the temporarily restrained beads into the sample solution compartment; (6) flowing the sample/bead mixture from the first separation station preferably to a second separation station having an actuatable attraction device; (7) actuating the second attraction device to attract some of the coated beads such that some of the coated beads are temporarily restrained within the second separation station; (8) flowing a wash solution compartment into the second separation station; (9) flowing a conjugate/carrier fluid solution compartment over the attracted beads; (10) actuating the second attraction device to release the temporarily restrained beads into the conjugate/carrier fluid solution compartment; (11) flowing the conjugate/bead mixture from the second separation station to a third separation station that preferably has a third actuatable attraction device having a sensor; (12) actuating the third actuatable attraction device to attract some of the beads, or more specifically, some of the bead/antigen/antibody/enzyme complex, to the vicinity of the sensor; (13) flowing a wash solution compartment into the third separation station; (14) flowing a substrate/carrier fluid solution compartment over the attracted beads, which

in the presence of the enzyme is cleaved into a reporter molecule capable of exhibiting redox recycling, and (15) measuring the presence or amount of electrochemical with the sensor, wherein the sensor produces redox recycling of the electrochemical.

Thus, devices and methods for detecting and quantitating analytes in a sample are disclosed. While embodiments and applications of this invention have been shown and described, it will be apparent to those skilled in the art that many modifications are possible without departing from the inventive concepts herein. The invention, therefore is not to be restricted except in the spirit of the appended claims.

We claim:

1. An electrochemical reporter device comprising:
   (a) a chamber for receiving an analytical reaction including magnetic beads;
   (b) a sensor within the chamber, the sensor for detecting electrochemical reporter molecules within the chamber and the sensor having a configuration such that reporter molecules capable of exhibiting redox recycling will undergo redox recycling if within the chamber; and
   (c) an actuatable magnetic field generating device selectively positioned such that magnetic beads present within the chamber will be attracted to the surface of the chamber wherein the sensor is located.

2. The electrochemical reporter device of claim 1, the sensor being a microelectronic interdigitated array of electrodes with a distance between the electrodes of about 100 to about 800 nanometers.

3. The electrochemical reporter device of claim 2, the sensor being a microelectronic interdigitated array of electrodes having a distance between the electrodes of about 300 nanometers.

4. An electrochemical reporter system comprising:
   (a) a magnetic bead;
   (b) a first recognition molecule capable of specifically binding an analyte in a structure restricted manner, the recognition molecule being linked to the magnetic bead;

(c) an enzyme;

(d) a coupling element, for coupling with specificity the enzyme to the recognition molecule/analyte complex or the analyte;

(e) a substrate which in the presence of the enzyme is cleavable into a reporter molecule capable of exhibiting redox recycling;

(f) a sensor for detecting the electrochemical reporter molecule, said sensor having a configuration such that the reporter molecule will exhibit redox recycling; and

(g) a magnetic field generating device positionable such that the magnetic beads may be attracted to the vicinity of the sensor.

5. The electrochemical reporter system of claim 4, the sensor being a microelectronic interdigitated array of electrodes with a distance between the electrodes of between about 100 to about 800 nanometers.

6. The electrochemical reporter system of claim 5, the distance between the electrodes being about 300 nanometers.

7. The electrochemical reporter system of claim 4, the enzyme being capable of effecting the cleavage of a covalent bond of the substrate.

8. The electrochemical reporter system of claim 7, the enzyme being selected from the group consisting of α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase, β-mannosidase, acid phosphatase, alkaline phosphatase and phosphodiesterase II.

9. The electrochemical reporter system of claim 4, the substrate being selected from the group consisting of p-aminophenyl-β-D-galactopyranoside, p-aminophenyl-α-D-galactopyranoside, p-aminophenyl-α-D-glucopyranoside, p-aminophenyl-β-D-glucopyranoside, p-aminophenyl-α-D-mannopyranoside, p-aminophenyl-β-D-mannopyranoside, p-aminophenylphosphate, and p-aminophenylphosphorylcholine.

10. The electrochemical reporter system of claim 4, the first recognition molecule being selected from the group consisting of a protein, a polypeptide, a nucleic acid, a nucleic acid analog, a hapten, immunoglobulin, fragments of immunoglobulin, non-immunoglobulin binding proteins, cell adhesion molecules, receptors, non-biologic binding molecules and a hormone.

11. The electrochemical reporter system of claim 4, the coupling element comprising a second recognition molecule coupled to an enzyme, the second recognition molecule being selected from the group consisting of a protein, a polypeptide, a nucleic acid, a nucleic acid analog, a hapten, immunoglobulin, fragments of immunoglobulin, non-immunoglobulin binding proteins, cell adhesion molecules, receptors, non-biologic binding molecules and a hormone.

12. The electrochemical reporter system of claim 4, the substrate being cleaved into at least one component comprising para-aminophenol.

13. The electrochemical reporter system of claim 4, the sensor being a microelectronic interdigitated array of electrodes having width between about 100 and about 800 nanometers and spaced between about 100 and about 800 nanometers from each other.

14. An assay for detecting or quantitating a specific analyte in a sample comprising the steps of:

a) a primary incubation, wherein magnetic beads coated with a first recognition molecule that specifically binds an analyte are incubated with a sample;

b) a secondary incubation, wherein the magnetic beads are incubated with a conjugate comprising an enzyme and a second recognition molecule that specifically binds the analyte, or the analyte/recognition molecule complex;

c) capturing the magnetic beads with a magnet over a sensor capable of producing redox recycling of an electrochemical capable of undergoing redox recycling;

d) adding a substrate, said substrate in the presence of the enzyme being cleaved into an electrochemical capable of undergoing redox recycling; and

e) detecting the presence or measuring the amount of electrochemical present in the solution with said sensor.

15. The assay of claim 14, the primary incubation lasting less than 10 minutes.

16. The assay of claim 14, the secondary incubation lasting less that 10 minutes.

17. The electrochemical reporter system of claim 14, the first recognition molecule being selected from the group

consisting of a protein, a polypeptide, a nucleic acid, a nucleic acid analog, a hapten, immunoglobulin, fragments of immunoglobulin, non-immunoglobulin binding proteins, cell adhesion molecules, receptors, non-biologic binding molecules and a hormone.

18. The electrochemical reporter system of claim 14, the second recognition molecule being selected from the group consisting of a protein, a polypeptide, a nucleic acid, a nucleic acid analog, a hapten, immunoglobulin, fragments of immunoglobulin, non-immunoglobulin binding proteins, cell adhesion molecules, receptors, non-biologic binding molecules and a hormone.

19. The electrochemical reporter system of claim 14, the enzyme being capable of effecting the cleavage of a covalent bond of the substrate.

20. The electrochemical reporter system of claim 19, the enzyme being selected from the group consisting of α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase, β-mannosidase, acid phosphatase, alkaline phosphatase and phosphodiesterase II.

21. The electrochemical reporter system of claim 14, the substrate being selected from the group consisting of p-aminophenyl-β-D-galactopyranoside, p-aminophenyl-α-D-galactopyranoside, p-aminophenyl-α-D-glucopyranoside, p-aminophenyl-β-D-glucopyranoside, p-aminophenyl-α-D-mannopyranoside, p-aminophenyl-β-D-mannopyranoside, p-aminophenylphosphate, and p-aminophenylphosphorylcholine.

22. The electrochemical reporter system of claim 14 wherein the substrate is cleaved into at least one component comprising para-aminophenol.

23. The electrochemical reporter system of claim 14 wherein the sensor is a microelectronic interdigitated array of electrodes having width between about 100 and about 800 nanometers and spaced between about 100 and about 800 nanometers from each other.

24. An electrochemical immunoassay for detecting an analyte in a sample comprising the steps of:

(a) having linked to a magnetic bead an antigen with an antibody specific for an analyte bound to the antigen, the antibody being coupled to an enzyme or having a coupling element for being specifically coupled to an enzyme;

(b) contacting the magnetic bead/antigen/antibody/enzyme complex with a sample to be analyzed;

(c) collecting the magnetic bead/antigen/antibody/enzyme complex;

(d) attracting the magnetic bead/antigen/antibody/enzyme complex to the vicinity of a sensor;

(e) adding a substrate to the collected magnetic bead/antigen/antibody/enzyme complex, the substrate in the presence of the enzyme being cleavable into a reporter molecule capable of exhibiting redox recycling; and

(f) measuring the presence or amount of reporter molecule with the sensor, the sensor being an

interdigitated array of electrodes capable of producing redox recycling of the reporter molecule.

25. An electrochemical assay for detecting a specific analyte in a sample comprising the steps of:

(a) having a recognition molecule linked to a magnetic bead, said recognition molecule capable of specifically binding the analyte in a structure restricted manner;

(b) contacting the magnetic bead with a sample to be analyzed;

(c) coupling with specificity an enzyme to the recognition molecule or the analyte;

(d) attracting the magnetic bead/recognition molecule/analyte/enzyme conjugate complex to the vicinity of a sensor with a device capable of generating a magnetic field;

(e) adding a substrate, which in the presence of the enzyme is cleaved into a reporter molecule capable of exhibiting redox recycling; and

(f) measuring the presence or amount of electrochemical with the sensor, wherein the sensor is an interdigitated array of electrodes capable of producing redox recycling of the electrochemical.

26. A kit for detecting or measuring an analyte in a sample the kit comprising

i) magnetic beads pre-coated with a first recognition molecule specific for the analyte;

ii) a second recognition molecule, specific for the analyte or the first recognition molecule/analyte complex, the second recognition molecule being conjugated to an enzyme;

iii) a substrate which in the presence of the enzyme generates an electrochemical capable of redox recycling.

27. The kit of claim 26 further comprising

iv) a single use electrochemical sensor module.

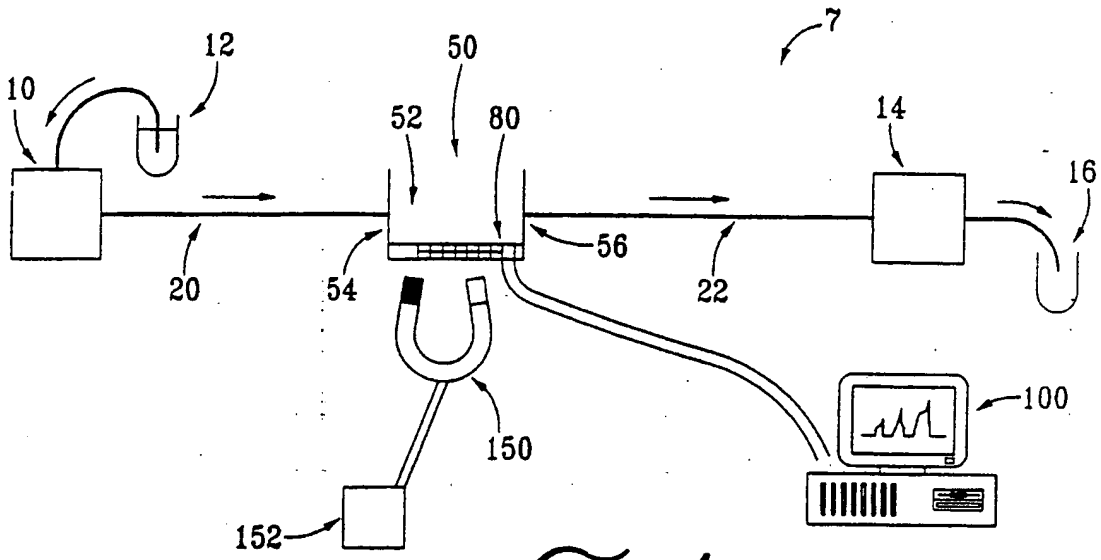


Fig. 1

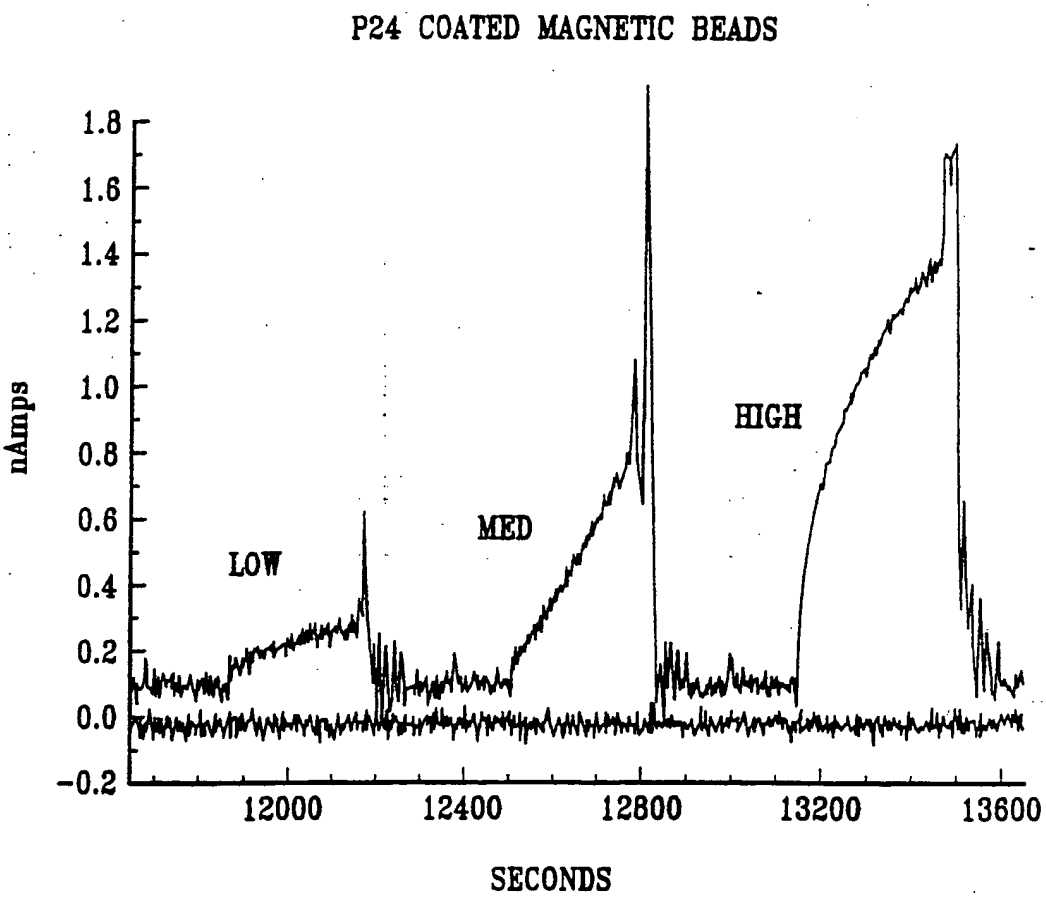

P24 COATED MAGNETIC BEADS
nAmps
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
LOW
MED
HIGH
12000
12400
12800
13200
13600
SECONDS

Fig. 2

	SAMPLE (mIU/mL)	+20 TO +80 SEC WINDOW SLOPE (nA/SEC)	+20 S THRU END OF CURVE SLOPE (nA/SEC)	
SET B	0	-0.0003	nA	
	15	0.0002	0.0001	SENSITIVITY -TO 15 mIU/mL FOR QUALITATIVE RESULT.
	50	0.0002	0.0002	
	100	0.0005	0.0002	
	200	0.0008	0.0004	-TO 50 mIU/mL FOR SEMI-QUANTITIVE RESULT.
	400	0.0011	0.0007	
	800	0.0016	0.0012	

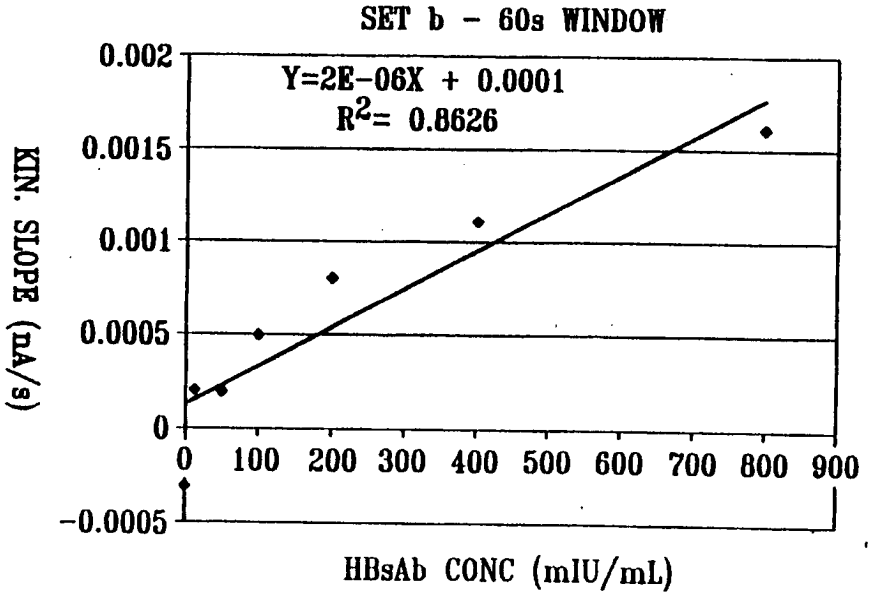
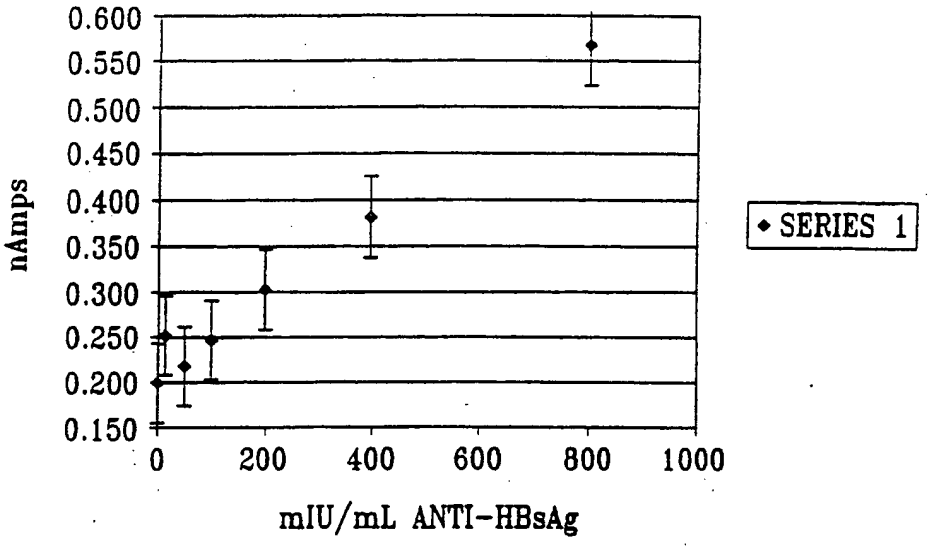


FIG. 3

FIG. 4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0.550
0.500
0.450
0.400
0.350
0.300
0.250
0.200
0.150
nAmps
0
200
400
600
800
1000
mIU/mL ANTI-HBsAg
◆ SERIES 1

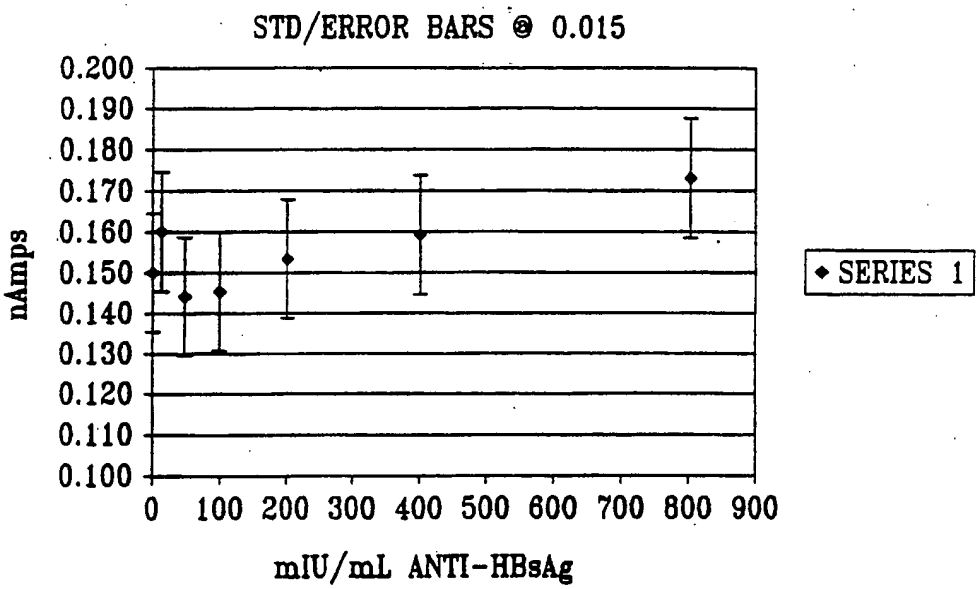

STD/ERROR BARS @ 0.015
0.200
0.190
0.180
0.170
0.160
0.150
0.140
0.130
0.120
0.110
0.100
nAmps
0
100
200
300
400
500
600
700
800
900
mIU/mL ANTI-HBsAg
◆ SERIES 1

FIG. 5

HBsAb (mIU/ml)	$5x10^6$ DATA (nA)	$0.8x10^6$ DATA (nA)
2000	0.3439	0.1472
500	0.2158	0.052
100	0.118	0.0177

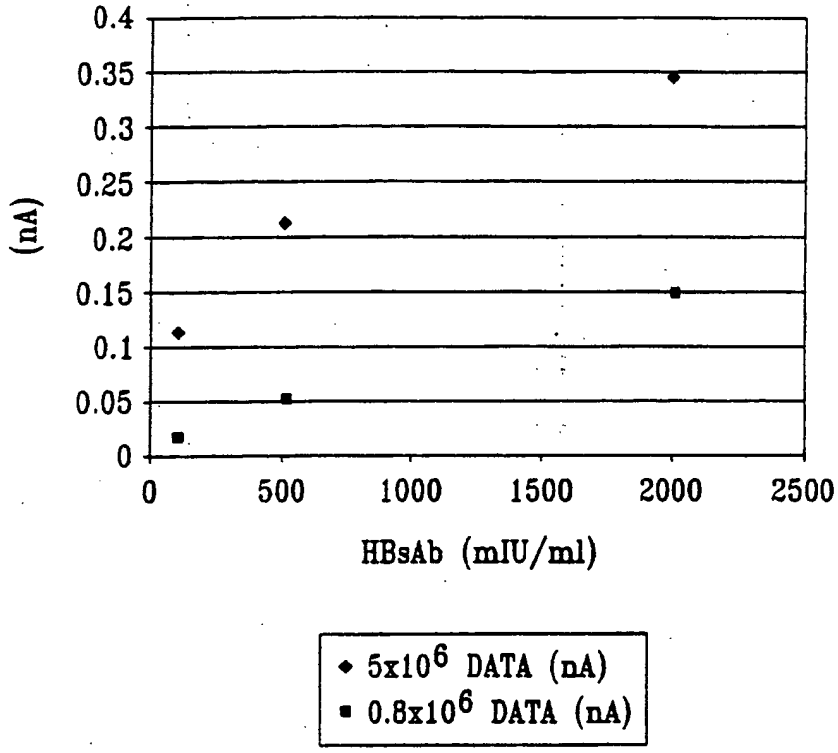


FIG. 6

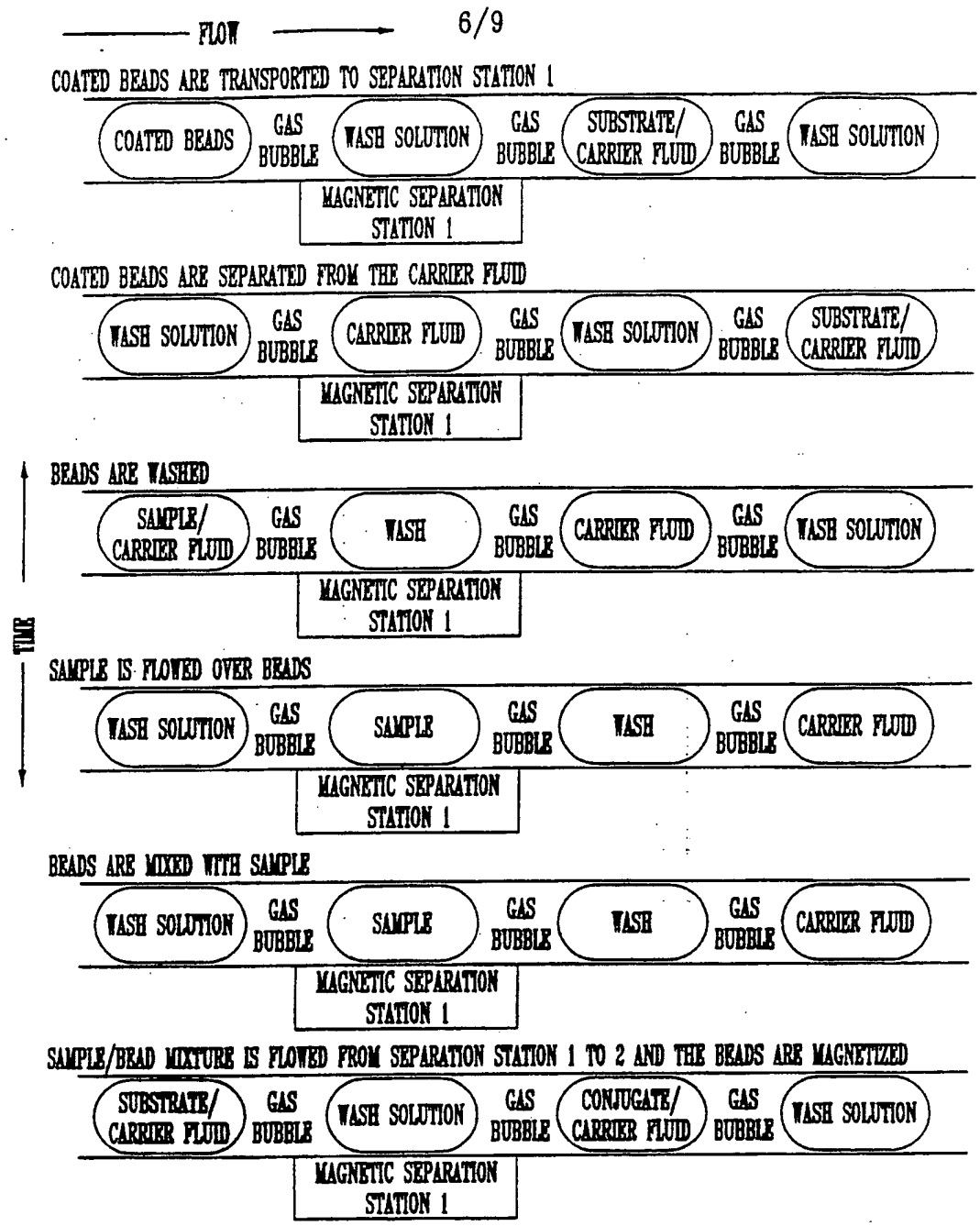
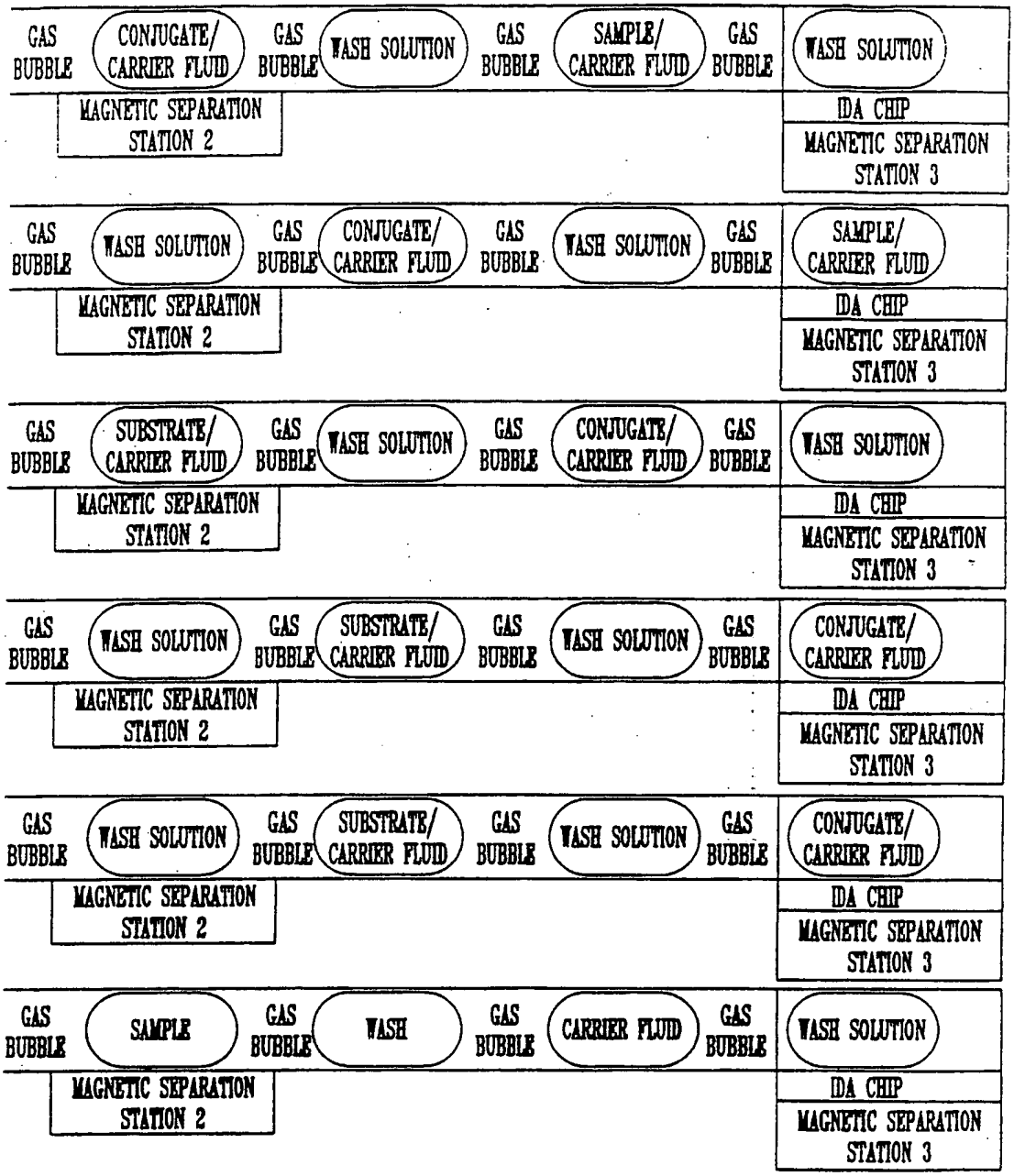


FIG. 7A

FIG. 7

FIG.7A	FIG.7B
FIG.7C	FIG.7D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SAMPLE/ CARRIER FLUID
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WASH SOLUTION
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IDA CHIP
MAGNETIC SEPARATION STATION 3
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WASH SOLUTION
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IDA CHIP
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IDA CHIP
MAGNETIC SEPARATION STATION 3
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CARRIER FLUID
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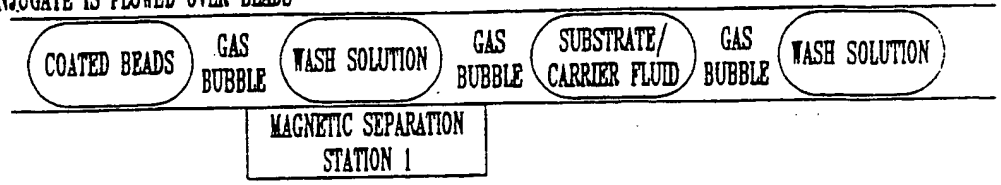
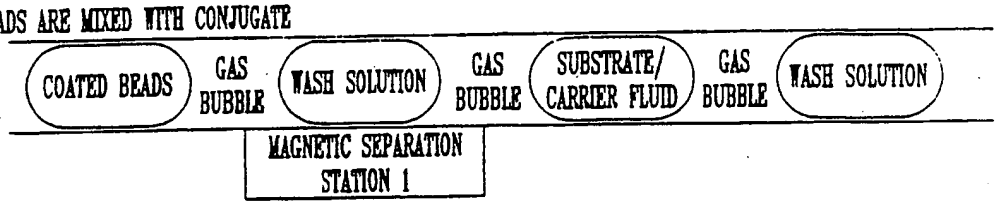
WASH SOLUTION
MAGNETIC SEPARATION STATION 2
IDA CHIP
MAGNETIC SEPARATION STATION 3

FIG. 7B

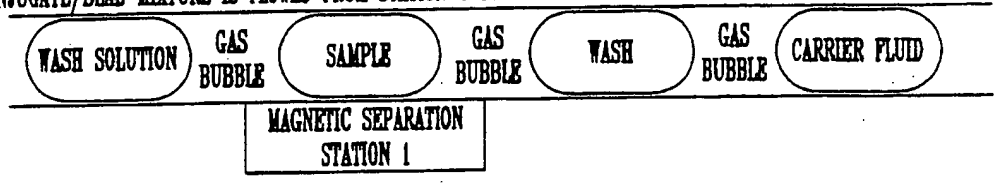
CONJUGATE IS FLOWED OVER BEADS



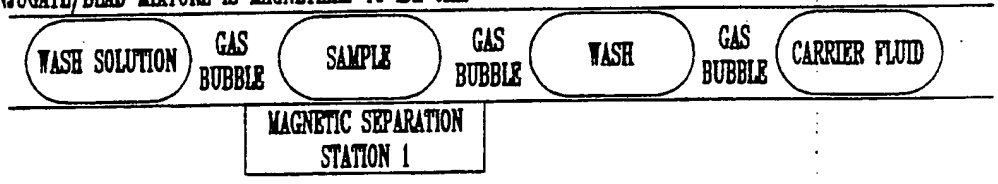
BEADS ARE MIXED WITH CONJUGATE

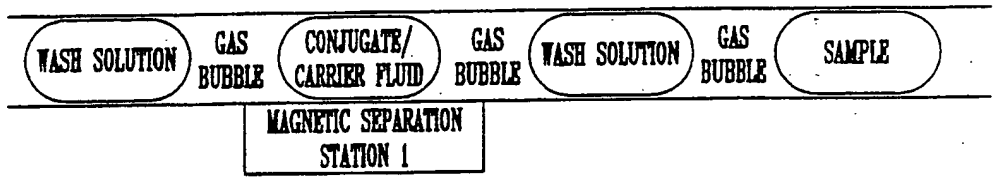


CONJUGATE/BEAD MIXTURE IS FLOWED FROM STATION 2 TO 3 OVER IDA CHIP AND THE BEADS ARE MAGNETIZED



CONJUGATE/BEAD MIXTURE IS MAGNETIZED TO IDA CHIP





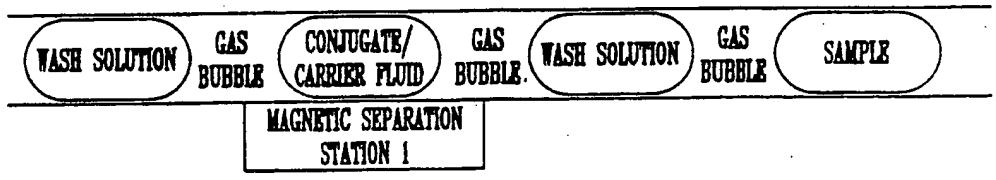
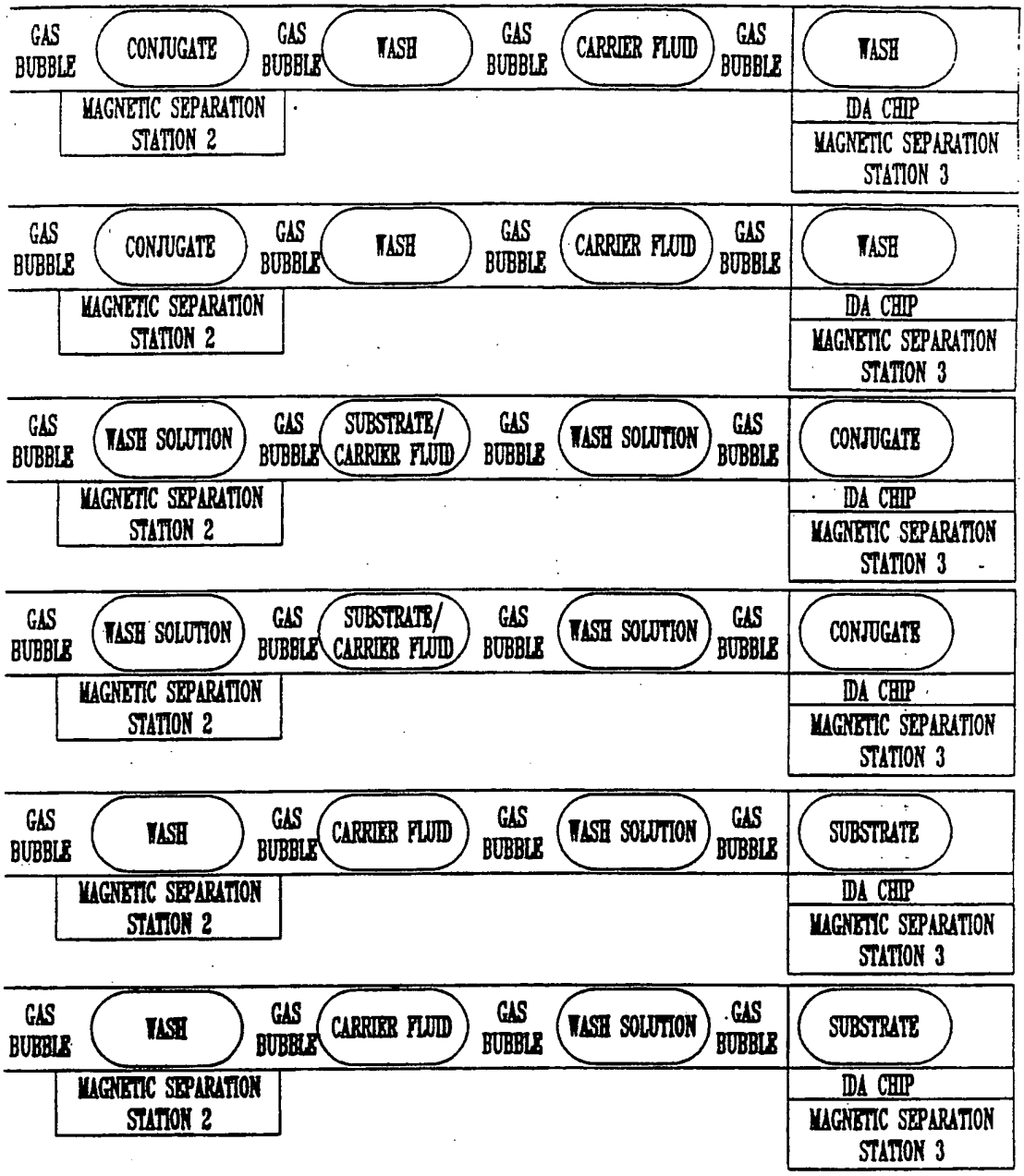


FIG. 7C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CARRIER FLUID
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WASH
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IDA CHIP
MAGNETIC SEPARATION STATION 3
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CONJUGATE
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WASH
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CARRIER FLUID
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IDA CHIP
MAGNETIC SEPARATION STATION 3
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IDA CHIP
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GAS BUBBLE
SUBSTRATE
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IDA CHIP
MAGNETIC SEPARATION STATION 3
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WASH
GAS BUBBLE
CARRIER FLUID
GAS BUBBLE
WASH SOLUTION
GAS BUBBLE
SUBSTRATE
MAGNETIC SEPARATION STATION 2
IDA CHIP
MAGNETIC SEPARATION STATION 3

FIG. 7D

INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/US 00/03485

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N27/327 G01N33/543 C12Q1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, COMPENDEX, BIOSIS, CHEM ABS Data, EMBASE, MEDLINE, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GEHRING A G ET AL: "Enzyme-linked immunomagnetic electrochemical detection of Salmonella typhimurium" JOURNAL OF IMMUNOLOGICAL METHODS,NL,ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, vol. 195, no. 1, 9 September 1996 (1996-09-09), pages 15-25, XP004021249 ISSN: 0022-1759	1,4, 7-12, 14-22, 26,27
Y	page 16, column 1, paragraph 1 -page 16, column 2, paragraph 2 figure 2	24,25

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 2000	13/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Muñoz, M

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 00/03485

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	O NIWA ET AL: "Small-volume voltammetric detection of 4-aminophenol with interdigitated array electrodes and its application to electrochemical enzyme immunoassay" ANALYTICAL CHEMISTRY,US,AMERICAN CHEMICAL SOCIETY. COLUMBUS, vol. 65, no. 11, 1 January 1993 (1993-01-01), pages 1559-1563, XP002082750 ISSN: 0003-2700 abstract page 1560, column 1, paragraph 2 ---	24,25
X	WEETALL H H ET AL: "A SIMPLE INEXPENSIVE DISPOSABLE ELECTROCHEMICAL SENSOR FOR CLINICAL AND IMMUNO-ASSAY" BIOSENSORS, vol. 3, no. 1, 1987, pages 57-64, XP000922945 ISSN: 0265-928X page 61, paragraph 2 -page 62, paragraph 1 figure 1 ---	1,4,7,8, 10,11, 14-20, 24-27
X	SANTANDREU M ET AL: "Development of electrochemical immunosensing systems with renewable surfaces" BIOSENSORS & BIOELECTRONICS, 1 JAN. 1998, ELSEVIER, UK, vol. 13, no. 1, pages 7-17, XP000922856 ISSN: 0956-5663 page 11 -page 12 figure 4 ---	1,4,7, 10,11, 14-19, 26,27
X	EP 0 859 229 A (GIST BROCADES BV) 19 August 1998 (1998-08-19) page 4, line 18 - line 28 page 4, line 49 - line 57 examples 4,5 ---	1,4,7,8, 10,11, 14-20, 26,27
X	WO 86 05815 A (GENETICS INT INC) 9 October 1986 (1986-10-09) claim 21 -----	1,4,10, 11, 14-18, 26,27

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INTERNATIONAL SEARCH REPORT

information on patent family members

Inter. nal Application No
PCT/US 00/03485

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 8605815 A	09-10-1986	AU 5667186 A	23-10-1986
		EP 0216844 A	08-04-1987